

Characteristics of Mild Dengue Virus Infection in Thai Children

In-Kyu Yoon,* Anon Srikiatkachorn, Laura Hermann, Darunee Buddhari, Thomas W. Scott, Richard G. Jarman, Jared Aldstadt, Ananda Nisalak, Suwich Thammaphalo, Piraya Bhoomiboonchoo, Mammen P. Mammen, Sharone Green, Robert V. Gibbons, Timothy P. Endy, and Alan L. Rothman

Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; University of Massachusetts Medical School, Worcester, Massachusetts; Department of Medicine, University of Toronto, Toronto, Ontario, Canada; Department of Entomology, University of California, Davis, Davis, California; Department of Geography, University at Buffalo, Buffalo, New York; Bureau of Epidemiology, Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand; Department of Infectious Diseases, State University of New York, Syracuse, Syracuse, New York; Institute for Immunology and Informatics, University of Rhode Island, Providence, Rhode Island; Fogarty International Center, National Institutes of Health, Bethesda, Maryland

Abstract. A four-year longitudinal cohort and geographic cluster study in rural Thailand was conducted to characterize the clinical spectrum of dengue virus (DENV) infection. Symptomatic DENV infections in the cohort were detected by active school absence–based surveillance that triggered cluster investigations around ill cohort children. Data from 189 cohort children with symptomatic DENV infection and 126 contact children in the clusters with DENV infection were analyzed. Of infected contacts, only 19% were asymptomatic; 81% were symptomatic, but only 65.9% reported fever. Symptom-based case definitions were unreliable for diagnosis. Symptomatic infections in contacts were milder with lower DENV RNA levels than the cohort. Infections in contacts with fever history were more likely to have detectable DENV RNA than infections without fever history. Mild infections identified by cluster investigations account for a major proportion of all DENV infections. These findings are relevant for disease burden assessments, transmission modeling, and determination of vaccine impact.

INTRODUCTION

Dengue virus (DENV) causes more human morbidity and mortality globally than any other vector-borne viral disease. Each year, an estimated 390 million persons are infected with DENV, of which 96 million are clinically apparent.¹ Most of the information about the clinical presentation of dengue illness comes from moderate-to-severe infections that prompt patients to seek medical care, providing the basis for the 1997 and 2009 World Health Organization (WHO) guidelines for dengue diagnosis and management.^{2–9} The sensitivities and specificities of these clinical indicators in identifying dengue illness are not well established. In particular, clinically mild DENV infections have not been as well described; information that is available has been obtained from prospective cohort studies and not from cluster studies, which can potentially detect milder illnesses than cohort studies as well as pre-symptomatic infections.^{10–15}

Symptomatic DENV infections can be difficult to distinguish from other febrile illnesses by using clinical parameters, especially with mild illness and early in the course of infection.^{8,16–18} A better understanding of the clinical and virologic characteristics across a wider clinical range of DENV infection is important to more accurately assess the burden of DENV infections and potential for virus transmission, and to make informed assessments of patients suspected of having dengue illness. We therefore conducted a combined longitudinal cohort and geographic cluster study in rural Thailand to evaluate the full clinical spectrum of DENV infection, including mild infections detected by cluster investigations. Such mild infections have not been previously well studied.^{12,19}

METHODS

Ethics statement. The study protocol was approved by the Institutional Review Boards of the Thai Ministry of Public Health, Walter Reed Army Institute of Research, University of Massachusetts Medical School, University of California, Davis, and San Diego State University. Written informed consent was obtained from the parents of study participants; assent was obtained from persons more than seven years of age.

Prospective longitudinal cohort and geographic cluster study. The study was conducted at 11 primary schools and 32 associated villages in rural areas of Muang District, Kamphaeng Phet Province in north central Thailand. The methods used are described elsewhere.^{12,19} During 2004–2007, a dynamic prospective longitudinal cohort of approximately 2,000 primary school children 4–15 years of age was monitored by active school absence–based surveillance during June–November each year.^{19,20} An acute-phase blood sample was drawn from cohort children who were absent from school and reported a fever in the previous seven days or had a measured temperature $\geq 38^{\circ}\text{C}$. A convalescent-phase blood sample was drawn 14 days later. A questionnaire assessing 12 specific symptoms was administered during the acute-phase and convalescent-phase visits; these symptoms could have been present at any time from seven days before the acute illness visit up to the day of the convalescent visit.

Acute-phase blood samples were tested by semi-nested reverse transcription–polymerase chain reaction (PCR) for detection of DENV RNA as described.^{21,22} Paired acute-phase and convalescent-phase blood samples were tested by using an in-house DENV/Japanese encephalitis virus (JEV) IgM/IgG capture enzyme immunoassay (EIA). Japanese encephalitis virus, which is endemic to rural Thailand, was included to rule out cross-reactivity with DENV.²³

Cohort children who were DENV PCR positive for an acute-phase blood sample collected within three days of illness onset served as an index cases for a positive geographic cluster

*Address correspondence to In-Kyu Yoon, Department of Virology, U.S. Army Medical Component, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, APO, AP, USA 96546. E-mail: yooni@afirms.org

Report Documentation Page				Form Approved OMB No. 0704-0188	
Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.					
1. REPORT DATE 01 DEC 2013		2. REPORT TYPE N/A		3. DATES COVERED -	
4. TITLE AND SUBTITLE Characteristics of mild dengue virus infection in thai children				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Yoon I. K., Srikiatkachorn A., Hermann L., Buddhari D., Scott T. W., Jarman R. G., Aldstadt J., Nisalak A., Thammapalo S., Bhoomiboonchoo P., Mammen M. P., Green S., Gibbons R. V., Endy T. P., Rothman A. L.,				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) United States Army Institute ofSurgical Research, JBSA Fort Sam Houston, TX				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release, distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 7	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

investigation around the child's house (although the index case may not have been the first infection in the cluster). Cohort children who were DENV PCR negative for an acute-phase blood sample served as an index cases for a negative (or control) geographic cluster investigation. Ten to 25 children six months to 15 years of age living within 100 meters of the index cases were enrolled in each cluster investigation regardless of presence or absence of symptoms. These contact children were evaluated on the day of enrollment (i.e., day 0), and 5, 10, and 15 days later by temperature measurement and administration of a symptom questionnaire similar to the cohort. Symptoms could have been present at any time from seven days before day 0 up to day 15. Blood samples were collected on days 0 and 15 and tested by DENV PCR and DENV/JEV IgM/IgG EIA. All DENV PCR-positive acute-phase samples from index cases and contact children also underwent quantitative reverse transcription PCR to determine serum viral RNA load at the time of blood collection.²⁴

Cohort children also underwent scheduled phlebotomy before the active surveillance season (i.e., May) and at the end of the surveillance season (i.e., December–January). Paired pre-/post-surveillance season blood samples were tested by hemagglutination inhibition (HAI) assay for all four DENV serotypes and JEV.²⁵ Samples with a four-fold increase in HAI titers were re-tested by serotype-specific plaque reduction neutralization tests (PRNT) for DENV and JEV to confirm DENV seroconversion.²⁶

Clinical and serologic classification. In cohort children, an acute symptomatic DENV infection was considered to have occurred if a febrile illness was associated with a positive DENV IgM EIA and/or PCR result in the acute-phase or convalescent-phase blood sample. DENV IgM-positive cases were considered to be primary infections (i.e., first in that child) if the DENV IgM:IgG ratio was ≥ 1.8 , and secondary infections (i.e., second or more in that child) if the ratio was < 1.8 .²³ A cohort child was considered to have clinically inapparent DENV infection during a surveillance season if paired pre-/post-season blood samples showed a four-fold increase in DENV HAI results confirmed by PRNT, but no symptomatic DENV infection was identified during that period.²⁶

For contacts in geographic clusters, an acute DENV infection was considered to have occurred if the day 0 or day 15 blood sample was positive by DENV IgM/IgG EIA and/or PCR. These DENV-infected contacts could have either symptomatic infection (i.e., symptoms detected by questionnaire or fever by temperature measurement) or asymptomatic infection (i.e., no detected symptoms or fever).

For cohort and contact participants, symptomatic DENV infections that required hospitalization were classified as dengue fever (DF) or dengue hemorrhagic fever (DHF) according to the 1997 WHO case definitions.² Symptomatic DENV infections that did not require hospitalization were considered as non-hospitalized symptomatic DENV infections.

Statistical analyses. SPSS for Windows version 19 (SPSS Inc., Chicago, IL) and MedCalc version 12.4 software were used for analyses. Symptoms were compared between the various diagnostic groups by using the chi-square test or Fisher's exact test for categorical variables or *t*-test for continuous variables. Variables significant in univariate analyses were subsequently entered in a logistic binary regression model to identify independent associations. A statistical level of $P < 0.05$ was considered significant.

RESULTS

Clinical and virologic features of DENV infection in the longitudinal cohort. There were 189 symptomatic DENV infections and 346 clinically inapparent DENV infections in the cohort. Twenty additional DENV infections were clinically unclassified because they had DENV HAI/PRNT seroconversion between pre-/post-season blood samples but with an acute febrile illness detected during active surveillance that did not have acute-phase/convalescent-phase blood samples collected.¹² General characteristics of dengue EIA-positive cohort children are shown in Table 1. By study design, all EIA-positive cohort children were symptomatic with a fever history. All four DENV serotypes were detected with a predominance of DENV-1 (46.9%) and DENV-4 (36.7%); secondary infection was much more frequent (93.1%) than primary infection. There were 40 hospitalized DENV infections (31 DF and 9 DHF) accounting for 21.2% of symptomatic DENV infections and 7.2% of all DENV infections (symptomatic plus inapparent/unclassified). By univariate analysis, a measured temperature $\geq 38.0^\circ\text{C}$, headache, anorexia, muscle/joint pain, rash, drowsiness, abdominal pain, diarrhea, and bleeding were significantly more frequent in DENV-infected cohort children with fever history than in non-DENV infected cohort children with fever history Table 2.

The two symptoms with the highest odds ratios in dengue versus non-dengue febrile illnesses, namely rash and bleeding, occurred infrequently (4.2% and 3.2% of symptomatic DENV infections, respectively). Cough and rhinorrhea were significantly more common in non-dengue illnesses than in dengue illnesses, but still occurred quite frequently with dengue (42.3% with cough and 23.8% with rhinorrhea in dengue). Logistic regression analysis showed that five clinical features (measured temperature $\geq 38.0^\circ\text{C}$, anorexia, rash, drowsiness, and bleeding) were independently associated with dengue febrile illnesses, and cough was associated with non-dengue illnesses (Table 2). No significant differences in symptoms were found between primary and secondary infections.

Fever history along with two or more symptoms from the 1997 WHO case definition for suspected DF (i.e., headache, muscle/joint pain, rash and bleeding) had moderate specificity for detecting symptomatic DENV infection (83.7%; 95%

TABLE 1
Characteristics of DENV-infected cohort and contact children, Thailand*

Description	DENV-infected cohort children, n = 189	DENV-infected contacts, n = 126†
Median age (range)	9 (5–13)	9 (0–15)
Sex		
M	92 (48.7)	68 (54.0)
F	97 (51.3)	58 (46.0)
Fever history	189 (100)	83 (65.9)
Serologic category		
Primary	13 (6.9)	23 (18.3)‡
Secondary	176 (93.1)	103 (81.7)‡
Dengue PCR positive	147 (77.8)	43 (34.1)
Virus serotype		
DENV-1	69 (46.9)	22 (51.1)
DENV-2	20 (13.6)	3 (7.0)
DENV-3	4 (2.7)	2 (4.7)
DENV-4	54 (36.7)	16 (37.2)

*Values are no. (%) unless otherwise indicated. DENV = dengue virus; PCR = polymerase chain reaction.

†Includes 119 contacts from positive clusters and 7 from negative clusters.

‡Variable timing of blood collection after infection in contacts makes this categorization less certain.

TABLE 2

Comparison of symptoms in DENV-infected and non-infected cohort children with fever history by univariate analysis (odds ratio) and binary logistic regression (adjusted odds ratio), Thailand*

Symptom	DENV-infected with fever history (%), n = 189	Non-DENV infected with fever history (%), n = 2449	Odds ratio (95% CI)	Adjusted odds ratio (95% CI)	P for adjusted odds ratio
Temperature $\geq 38^{\circ}\text{C}$	125 (66.1)	1,374 (56.1)	1.5 (1.1–2.1)	1.6 (1.1–2.2)	0.007
Headache	160 (84.7)	1,872 (76.4)	1.7 (1.1–2.6)	–	NS
Anorexia	61 (32.3)	473 (19.3)	2.0 (1.4–2.8)	1.7 (1.2–2.5)	0.002
Nausea/vomiting	61 (32.3)	673 (27.5)	1.3 (0.9–1.7)	–	NS
Muscle/joint pain	49 (25.9)	446 (18.2)	1.6 (1.1–2.2)	–	NS
Rash	8 (4.2)	19 (0.8)	5.7 (2.4–13.1)	4.3 (1.7–10.4)	0.002
Rhinorrhea	58 (30.7)	1,163 (47.5)	0.5 (0.4–0.7)	–	NS
Cough	80 (42.3)	1,637 (66.8)	0.4 (0.3–0.5)	0.4 (0.3–0.5)	< 0.001
Drowsiness	45 (23.8)	278 (11.4)	2.4 (1.7–3.5)	2.0 (1.4–2.9)	< 0.001
Abdominal pain	46 (24.3)	377 (15.4)	1.8 (1.3–2.5)	–	NS
Diarrhea	14 (7.4)	92 (3.8)	2.1 (1.1–3.7)	–	NS
Bleeding (any site)	6 (3.2)	15 (0.6)	5.3 (2.0–13.9)	4.5 (1.6–12.4)	0.004

*DENV = dengue virus; CI = confidence interval; NS = not significant.

confidence interval [CI] = 82.2–85.2), but low sensitivity (27.5%; 95% CI = 21.3–34.5). The positive and negative predictive values for this symptom complex were 11.5% (95% CI = 8.7–14.8) and 93.7% (95% CI = 92.7–94.7), respectively. These values were similar if symptoms from the 2009 WHO criteria for probable dengue illness were used (i.e., fever with two or more of nausea/vomiting, rash, muscle/joint pain, abdominal pain, drowsiness [used as a surrogate for lethargy], or bleeding). Specificity was 81.2% (95% CI = 79.6–82.8), sensitivity was 32.3% (95% CI = 25.7–39.4), positive predictive value was 11.7% (95% CI = 9.1–14.8), and negative predictive value was 94.0% (95% CI = 92.9–95.0).

Some symptom combinations increased the likelihood of a cohort illness being caused by DENV infection (e.g., anorexia and bleeding [odds ratio (OR) = 13.2, 95% CI = 3.3–53.3] or rash and absence of cough [OR = 8.99, 95% CI = 3.1–25.3]). However, these symptom combinations were uncommon (< 10% of symptomatic DENV infections). Other symptom combinations, including various combinations of headache, anorexia and measured temperature $\geq 38.0^{\circ}\text{C}$, also yielded moderate sensitivities but were common in non-dengue febrile illnesses.

Drowsiness and bleeding history (e.g., bleeding gums, epistaxis, hematemesis, hematochezia, or melena) were the only symptoms individually associated with disease severity. Both symptoms were more common in DHF than in DF (OR = 7.2, 95% CI = 1.7–30.2 for drowsiness and OR = 29.5, 95% CI = 4.9–177.6 for bleeding) and in hospitalized compared with non-hospitalized dengue illnesses (OR = 5.0, 95% CI = 2.3–10.5 and OR = 8.2, 95% CI = 1.4–46.3, respectively).

The proportion of symptomatic DENV infections that were DENV PCR positive varied with the duration between illness onset and acute-phase blood collection. The PCR-positive rate among symptomatic DENV infections was > 80% when the acute-phase blood sample was collected within three days of illness onset and decreased to 64% after the third day of illness. The DENV PCR result was positive in 143 (86%) of 166 DENV infections when the acute-phase blood sample was DENV IgM negative, and in 4 (22%) of 18 symptomatic DENV infections when the acute-phase blood sample was IgM positive ($P < 0.001$, by chi-square test).

Clinical and virologic features of DENV infection in geographic clusters. In 50 positive cluster investigations, 119 (14.8%) of 805 contact children had laboratory-confirmed acute DENV infection on day 0. An additional 10 contacts who had

DENV infection based solely on day 15 PCR-positive results were not included in further analysis because no clinical information was available after day 15. In 53 negative clusters, 7 (0.9%) of 794 contacts had acute DENV infection; an additional two contacts were PCR positive on day 15, but were not included in further analysis.¹² General characteristics of DENV-infected contacts in the geographic clusters are shown in Table 1. All four DENV serotypes were recovered with the same two serotypes predominating as in the cohort: DENV-1 (51.1%) and DENV-4 (37.2%); secondary infection (81.7%) was more frequent than primary infection but slightly less so than in the cohort. Asymptomatic DENV infections were detected in the clusters: 24 (19.0%) of 126 DENV infections were asymptomatic and 102 (81.0%) were symptomatic. Of the symptomatic infections, 19 (18.6%) did not have fever history and reported no antipyretic use. Seven DENV-infected contacts were hospitalized with DF or DHF (all from positive clusters) accounting for 7.0% of symptomatic infections and 5.6% of total DENV infections (symptomatic plus asymptomatic).

Clinical symptoms in the 83 DENV-infected contacts who reported fever are shown in Table 3. The comparison group includes DENV-negative contacts who reported fever in positive and negative clusters; DENV-negative febrile contacts in positive versus negative clusters had no significant differences in symptoms. Univariate analysis showed that symptoms of headache, anorexia, nausea/vomiting, muscle/joint pain, rash, abdominal pain, and bleeding were more frequent in DENV-infected febrile contacts than in DENV-negative febrile contacts. Logistic regression analysis showed that headache, muscle/joint pain, and rash were independently associated with DENV infection (Table 3). Rash had the strongest association (adjusted OR [AOR] = 7.6, 95% CI = 3.0–19.8), but was present in only 15.7% of DENV-infected febrile contacts. Comparing primary with secondary symptomatic DENV infections, we showed that nausea and rash were more common in primary infection (AOR = 18.2, 95% CI 1.8–186.7 and AOR = 13.9, 95% CI = 2.0–97.5, respectively) whereas headache was less common in primary infection (AOR = 0.2, 95% CI = 0.01–0.3). Comparing symptomatic DENV-infected contacts with and without fever history showed that those without fever had fewer symptoms (not including fever) than those with fever Table 4. Headache and nausea/vomiting were significantly more common in DENV-infected contacts with fever than in symptomatic DENV-infected contacts without fever. None of the 19 symptomatic DENV-infected

TABLE 3

Comparison of symptoms in DENV-infected and non-infected contacts with fever history by univariate analysis (odds ratio) and binary logistic regression (adjusted odds ratio), Thailand*

Symptom	DENV-infected with fever history (%), n = 83	Non-DENV infected with fever history (%), n = 455	Odds ratio (95% CI)	Adjusted odds ratio (95% CI)	P for adjusted odds ratio
Temperature $\geq 38^{\circ}\text{C}$	29 (34.9)	165 (36.3)	0.9 (0.6–1.5)	–	NS
Headache	50 (60.2)	159 (34.9)	2.9 (1.8–4.6)	2.1 (1.3–3.5)	0.004
Anorexia	20 (24.1)	52 (11.4)	2.5 (1.4–4.4)	–	NS
Nausea/vomiting	26 (31.3)	65 (14.3)	2.7 (1.6–4.7)	–	NS
Muscle/joint pain	13 (15.7)	18 (4.0)	4.5 (2.1–9.6)	2.8 (1.2–6.5)	0.01
Rash	13 (15.7)	8 (1.8)	10.4 (4.2–25.9)	7.6 (3.0–19.8)	< 0.001
Rhinorrhea	40 (48.2)	239 (52.5)	0.8 (0.5–1.3)	–	NS
Cough	44 (53.0)	232 (51.0)	1.1 (0.7–1.7)	–	NS
Drowsiness	9 (10.8)	25 (5.5)	2.1 (0.9–4.7)	–	NS
Abdominal pain	17 (20.5)	42 (9.2)	2.5 (1.4–4.7)	–	NS
Diarrhea	5 (6.0)	25 (5.5)	1.1 (0.4–3.0)	–	NS
Bleeding (any site)	6 (7.2)	10 (2.2)	3.5 (1.2–9.8)	–	NS

*DENV = dengue virus; CI = confidence interval; NS = not significant.

contacts without fever reported any symptoms of nausea/vomiting, muscle/joint pain, drowsiness, bleeding, or diarrhea.

Although we identified individual symptoms that distinguished dengue from non-dengue febrile illnesses in contacts, no combination of symptoms was able to distinguish between them. We were also unable to determine any symptom or symptom combination associated with dengue severity, although our analysis was limited by the small number of contacts with severe disease. Symptoms from the 1997 WHO case definition for suspected DF had high specificity for detecting febrile symptomatic DENV infection (94.7%, 95% CI = 92.3–96.6), but low sensitivity (27.7%, 95% CI = 18.5–38.6). Positive and negative predictive values were 48.9% (95% CI = 34.1–63.9) and 87.8% (95% CI = 84.6–90.5), respectively. Values were similar using symptoms from the 2009 WHO criteria for probable dengue illness with high specificity (91.7%, 95% CI = 88.7–94.0) and low sensitivity (28.9%, 95% CI = 19.5–39.9); positive and negative predictive values were 38.7% (95% CI = 26.6–51.9) and 87.6% (95% CI = 84.3–90.4), respectively.

For symptomatic DENV infections among contacts in which day 0 blood samples were collected within three days of illness onset, 18 (58.1%) of 31 blood samples were PCR positive. The DENV PCR result was positive in 33 (60.0%) of 55 DENV infections when the day 0 blood sample was DENV IgM negative, but in only three (15.0%) of 20 DENV infections when the day 0 sample was IgM positive ($P < 0.001$, by chi-square test).

Dengue virus RNA levels in cohort and contact children. Comparing symptoms between DENV-infected cohort and

contact children, we found that cohort children were more likely to have a measured temperature $\geq 38^{\circ}\text{C}$, headache, muscle/joint pain, rash, and drowsiness, whereas contacts were more likely to have rhinorrhea and cough Table 5. Per study design, all 50 persons with index cases in positive clusters were DENV PCR positive for their acute-phase blood samples within three days of illness onset; DHF developed in three of these persons. Among contacts, 40 PCR-positive DENV infections were detected from day 0 blood samples, of which 18 were collected within three days of illness onset. All 18 of these contacts were symptomatic with fever; none had DHF. Comparing the 47 non-DHF dengue index cases with these 18 DENV PCR-positive contacts, we showed that the mean quantity of DENV RNA was 2.3×10^7 (range = 9.9×10^2 – 2.14×10^8) copies/mL in index cases versus 7.1×10^6 (range = 1.9×10^2 – 4.3×10^7) copies/mL in contacts ($P = 0.03$, by t test). Symptoms were not significantly different between these two groups.

DISCUSSION

Our combined cohort and cluster study demonstrates characteristics of DENV infection in children across a wide clinical spectrum of disease including mild infections identified from cluster investigations that have not been previously well described. Our results are applicable for disease burden assessments in endemic areas, virus transmission dynamics, clinical diagnosis, and disease pathophysiology. In our study

TABLE 4

Comparison of symptoms between DENV-infected contacts with and without fever history, Thailand*

Symptom	Symptomatic DENV-infected with fever history (%), n = 83†	Symptomatic DENV-infected without fever history (%), n = 19†	P
Temperature $\geq 38^{\circ}\text{C}$	29 (34.9)	0 (0.0)	0.001
Headache	50 (60.2)	3 (15.8)	< 0.001
Anorexia	20 (24.1)	2 (10.5)	NS
Nausea/vomiting	26 (31.3)	0 (0.0)	0.003
Muscle/joint pain	13 (15.7)	0 (0.0)	NS
Rash	13 (15.7)	1 (5.3)	NS
Rhinorrhea	40 (48.2)	9 (47.4)	NS
Cough	44 (53.0)	8 (42.1)	NS
Drowsiness	9 (10.8)	0 (0.0)	NS
Abdominal pain	17 (20.5)	4 (21.1)	NS
Diarrhea	5 (6.0)	0 (0.0)	NS
Bleeding (any site)	6 (7.2)	0 (0.0)	NS

*DENV = dengue virus; NS = not significant.

†38 of 83 with fever history and 2 of 19 without fever history were DENV polymerase chain reaction positive ($P = 0.004$, by Fisher's exact test).

TABLE 5
Comparison of symptoms between symptomatic DENV-infected cohort and contact children, Thailand*

Symptom	Symptomatic DENV-infected cohort children (%), n = 189	Symptomatic DENV-infected contacts (%), n = 102	P
Fever history	189 (100.0)	83 (81.4)	0.0001
Temperature $\geq 38^{\circ}\text{C}$	125 (66.1)	29 (28.4)	0.0001
Headache	160 (84.7)	53 (52.0)	0.0001
Anorexia	61 (32.3)	22 (21.6)	NS
Nausea/vomiting	61 (32.3)	26 (25.5)	NS
Muscle/joint pain	49 (25.9)	13 (12.7)	0.01
Rash	8 (4.2)	14 (13.7)	0.005
Rhinorrhea	58 (30.7)	49 (48.0)	0.005
Cough	80 (42.3)	52 (51.0)	0.0002
Drowsiness	45 (23.8)	9 (8.8)	0.002
Abdominal pain	46 (24.3)	21 (20.6)	NS
Diarrhea	14 (7.4)	5 (4.9)	NS
Bleeding (any site)	6 (3.2)	6 (5.9)	NS

*DENV = dengue virus; NS = not significant.

population, mild dengue illness accounted for most symptomatic DENV infections across all four serotypes.

Symptomatic DENV infections accounted for 81.0% of all DENV infections in contact children, but only 35.3% in cohort children. However, symptomatic infections seen in contacts were milder than in cohort children and fewer symptoms were reported. This finding may be explained by the difference in surveillance methods between the two groups. Contacts were evaluated for acute infection without regard to their clinical or functional status, whereas cohort children were only evaluated for acute infection if they missed school because of a febrile illness. Hospitalized children with DENV infections accounted for a similar percentage of all DENV infections in cohort and contact children (7.2% and 5.6%, respectively). Because it is likely that hospitalized children with infections would be detected no matter what the surveillance method, the fact these percentages were similar suggests that most DENV infections were detected in the cohort and clusters, although the sensitivity for detecting symptoms varied with the surveillance method. These findings demonstrate that mildly symptomatic DENV infections, including afebrile illnesses and mild febrile illnesses, identified only by cluster investigations, constitute a previously uncharacterized spectrum of symptomatic disease. This finding highlights the importance of the surveillance method in defining illness for purposes such as disease burden assessments, transmission modeling, and determination of vaccine impact.

Certain symptoms and symptom combinations were able to distinguish dengue from non-dengue illnesses. However, these symptoms or symptom combinations occurred infrequently in DENV infections. Cough was able to distinguish non-dengue from dengue illnesses, but occurred too frequently in dengue illness to be used to exclude the diagnosis. Abdominal pain is a warning sign from the 2009 WHO dengue guidelines requiring close observation and medical intervention, although these warning signs have not been validated with respect to sensitivity, specificity, and positive and negative predictive values. We noted no significant difference in abdominal pain between DENV-infected and -uninfected symptomatic children in the cohort and contact groups. Abdominal pain was not infrequent in mild DENV infections. Symptoms from the 1997 and 2009 WHO case definitions for suspected or probable DF had moderate-to-high specificity and negative predictive value in distinguishing mild dengue illness from non-dengue illnesses, although the sensitivity and positive predictive value were low.

In hyperendemic regions of Asia, these symptoms may have some limited utility in excluding DENV infection. However, this finding would depend on the identity and attack rates of other co-circulating pathogens such as influenza virus, chikungunya virus, or *Leptospira*. Our study did not determine the specific etiologies of non-dengue illnesses.

Considering only those symptomatic DENV infections not resulting in DHF, we found that serum DENV RNA levels were significantly higher in DENV PCR-positive index cases than in PCR-positive contacts in blood samples collected within three days of illness onset. Infections in contacts were generally milder than in cohort children with fewer reported symptoms. In addition, DENV infections in contacts with fever history were more likely to be PCR positive than those without fever history. The 19 DENV infections without fever history described in Table 4 were, for the most part, diagnosed only by serologic analysis. These were nevertheless still probably acute infections because other symptoms besides fever were present and given that few infections were comparably detected by serologic analysis in the negative clusters. Other studies have reported higher viral loads in persons with DHF than in those with DF.^{27,28} Our results suggest that serum viral load also differs with more subtle clinical differences in symptomatic outpatient dengue. Differing viral loads in outpatients has implications for the differential ability of infected humans to transmit virus to mosquitoes. The severity of disease across the entire clinical spectrum should be factored into models that seek to predict patterns in DENV transmission.

Our study was limited to children in a dengue hyperendemic area of rural Thailand. Therefore, we do not know how well these findings apply to other regions with different dengue epidemiology or to adult populations. In addition, our study design did not enable us to conduct detailed analyses of changes in clinical features during the course of dengue illness as has been reported for a longitudinal cohort in Nicaragua,¹⁶ and our use of a symptom questionnaire at intervals separated by several days to two weeks may have led to some recall bias. We also cannot exclude the possibility of enrollment bias in the cluster investigations, for example, if healthy children were less inclined to participate in the study than sick children. Nevertheless, data obtained from DENV-infected children from our cluster investigations provides unique information on mild illness that has largely been unavailable.

By combining data from a longitudinal cohort with cluster investigations, we show a wide clinical spectrum of DENV

infection in children that includes mild illnesses. Although mildly symptomatic DENV infection is difficult to distinguish from other febrile illnesses, it is quite common and these mild cases should be considered when characterizing DENV infection and transmission. In addition, detection of DENV infection through cluster studies may be useful in addressing pathophysiologic, immunologic, and clinical aspects of disease progression from very early in the course of infection.

Received July 23, 2013. Accepted for publication August 18, 2013.

Published online October 14, 2013.

Acknowledgments: We thank Dr. Chusak Pimgate, Dr. Chonticha Klungthong, Dr. Butsaya Thaisomboonsuk, Chaleaw Saengchan, Thanyalak Fansiri, Udom Kijchalao, and other clinical, laboratory, and entomological personnel of the Armed Forces Research Institute of Medical Sciences for their contributions; Dr. Kamchai Rungsimanphaiboon for his support of the field laboratory; the political, educational, medical, and community workers and leaders in Kamphaeng Phet, Thailand, for their support; and the children and parents involved in this study for their participation. This research benefited from discussions with working group members in the Research and Policy for Infectious Disease Dynamics program of the Science and Technology Directorate, U.S. Department of Homeland Security, and the Fogarty International Center, U.S. National Institutes of Health.

Financial support: This research was supported, in part, by the National Institutes of Health (grants P01 AI34533 and R01 GM083224), the U.S. Military Infectious Diseases Research Program (grant S0016-04-AF), the Bill and Melinda Gates Foundation Global Health Program (grant OPP52250), and the Canadian Institutes of Health Research Fellowship (Laura Hermann). The funding source had no role in the study design, data collection, analysis and interpretation, manuscript writing, or manuscript submission for publication.

Disclaimer: The views expressed in this article are those of the authors and do not represent the official policy or position of the U.S. Department of the Army, Department of Defense, or U.S. Government.

Authors' addresses: In-Kyu Yoon, Department of Virology, U.S. Army Medical Component, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, E-mail: yooni@afirms.org. Anon Srikiatkachorn, University of Massachusetts Medical School, Worcester, MA, E-mail: anon@afirms.org. Laura Hermann, Ananda Nisalak, Piraya Bhoomiboonchoo, and Robert V. Gibbons, Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, E-mails: laura.hermann@afirms.org, anandaN@afirms.org, pirayaB@afirms.org, and robert.gibbons@afirms.org. Darunee Buddhari, Kamphaeng Phet - AFRIMS Virology Research Unit, Muang District, Kamphaeng Phet, Thailand, E-mail: daruneet@afirms.org. Thomas W. Scott, Department of Entomology, University of California, Davis, CA, E-mail: twscott@ucdavis.edu. Richard G. Jarman, Viral Diseases Branch, Walter Reed Army Institute of Research, Silver Spring, MD, E-mail: richard.g.jarman.mil@mail.mil. Jared Aldstadt, Department of Geography, University at Buffalo, Buffalo, NY, E-mail: geojared@buffalo.edu. Suwich Thammaphalo, Bureau of Epidemiology, Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand, E-mail: sthammapalo@yahoo.com. Mammen P. Mammen, Vical Incorporated, San Diego, CA, E-mail: mammen.mammen@vical.com. Sharone Green, University of Massachusetts Medical School, Worcester, MA, E-mail: Sharone.Green@umassmed.edu. Timothy P. Endy, Department of Infectious Diseases, State University of New York, Syracuse, NY, E-mail: endyt@upstate.edu. Alan L. Rothman, Institute for Immunology and Informatics, University of Rhode Island, Providence, RI, E-mail: alan_rothman@mail.uri.edu.

REFERENCES

- Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, Drake JM, Brownstein JS, Hoen AG, Sankoh O, Myers MF, George DB, Jaenisch T, Wint GR, Simmons CP, Scott TW, Farrar JJ, Hay SI, 2013. The global distribution and burden of dengue. *Nature* 496: 504–507.
- WHO, 1997. *Dengue Haemorrhagic Fever: Diagnosis, Treatment, Prevention and Control*. 2nd edition. Geneva: World Health Organization.
- Special Programme for Research and Training in Tropical Diseases, World Health Organization, 2009. *Dengue: Guidelines for Diagnosis, Treatment, Prevention, and Control*. Geneva: TDR: World Health Organization.
- Nimmannitya S, Halstead SB, Cohen S, Margiotta MR, 1969. Dengue and chikungunya virus infection in man in Thailand, 1962–1964. I. Observations on hospitalized patients with hemorrhagic fever. *Am J Trop Med Hyg* 18: 954–971.
- Kalayanarooj S, Vaughn DW, Nimmannitya S, Green S, Suntayakorn S, Kunentrasai N, Viramitrachai W, Ratanachueke S, Kiatpolpoj S, Innis BL, Rothman AL, Nisalak A, Ennis FA, 1997. Early clinical and laboratory indicators of acute dengue illness. *J Infect Dis* 176: 313–321.
- Alexander N, Balmaseda A, Coelho IC, Dimaano E, Hien TT, Hung NT, Janisch T, Kroeger A, Lum LC, Martinez E, Siqueira JB, Thuy TT, Villalobos I, Villegas E, Wills B, 2011. Multicentre prospective study on dengue classification in four South-east Asian and three Latin American countries. *Trop Med Int Health* 16: 936–948.
- Potts JA, Gibbons RV, Rothman AL, Srikiatkachorn A, Thomas SJ, Supradish PO, Lemon SC, Libraty DH, Green S, Kalayanarooj S, 2010. Prediction of dengue disease severity among pediatric Thai patients using early clinical laboratory indicators. *PLoS Negl Trop Dis* 4: e769.
- Potts JA, Rothman AL, 2008. Clinical and laboratory features that distinguish dengue from other febrile illnesses in endemic populations. *Trop Med Int Health* 13: 1328–1340.
- Rocha C, Silva S, Gordon A, Hammond SN, Elizondo D, Balmaseda A, Harris E, 2009. Improvement in hospital indicators after changes in dengue case management in Nicaragua. *Am J Trop Med Hyg* 81: 287–292.
- Burke DS, Nisalak A, Johnson DE, Scott RM, 1988. A prospective study of dengue infections in Bangkok. *Am J Trop Med Hyg* 38: 172–180.
- Endy TP, Chunsuttiwat S, Nisalak A, Libraty DH, Green S, Rothman AL, Vaughn DW, Ennis FA, 2002. Epidemiology of inapparent and symptomatic acute dengue virus infection: a prospective study of primary school children in Kamphaeng Phet, Thailand. *Am J Epidemiol* 156: 40–51.
- Yoon IK, Rothman AL, Tannitisupawong D, Srikiatkachorn A, Jarman RG, Aldstadt J, Nisalak A, Mammen MP Jr, Thammaphalo S, Green S, Libraty DH, Gibbons RV, Getis A, Endy T, Jones JW, Koenraad CJ, Morrison AC, Fansiri T, Pimgate C, Scott TW, 2012. Underrecognized mildly symptomatic viremic dengue virus infections in rural Thai schools and villages. *J Infect Dis* 206: 389–398.
- Porter KR, Beckett CG, Kosasih H, Tan RI, Alisjahbana B, Rudiman PI, Widjaja S, Listiyaningsih E, Ma'Roef CN, McArdle JL, Parwati I, Sudjana P, Jusuf H, Yuwono D, Wuryadi S, 2005. Epidemiology of dengue and dengue hemorrhagic fever in a cohort of adults living in Bandung, West Java, Indonesia. *Am J Trop Med Hyg* 72: 60–66.
- Balmaseda A, Standish K, Mercado JC, Matute JC, Tellez Y, Saborio S, Hammond SN, Nunez A, Aviles W, Henn MR, Holmes EC, Gordon A, Coloma J, Kuan G, Harris E, 2010. Trends in patterns of dengue transmission over 4 years in a pediatric cohort study in Nicaragua. *J Infect Dis* 201: 5–14.
- Tien NT, Luxemburger C, Toan NT, Pollissard-Gadroy L, Huong VT, Van Be P, Rang NN, Wartel TA, Lang J, 2010. A prospective cohort study of dengue infection in schoolchildren in Long Xuyen, Viet Nam. *Trans R Soc Trop Med Hyg* 104: 592–600.
- Biswas HH, Ortega O, Gordon A, Standish K, Balmaseda A, Kuan G, Harris E, 2012. Early clinical features of dengue virus infection in Nicaraguan children: a longitudinal analysis. *PLoS Negl Trop Dis* 6: e1562.
- Kumar R, Tripathi P, Tripathi S, Kanodia A, Pant S, Venkatesh V, 2008. Prevalence and clinical differentiation of dengue fever in children in northern India. *Infection* 36: 444–449.
- Chadwick D, Arch B, Wilder-Smith A, Paton N, 2006. Distinguishing dengue fever from other infections on the basis of simple clinical and laboratory features: application of logistic regression analysis. *J Clin Virol* 35: 147–153.

19. Mammen MP, Pimgate C, Koenraadt CJ, Rothman AL, Aldstadt J, Nisalak A, Jarman RG, Jones JW, Srikiatkachorn A, Ypil-Butac CA, Getis A, Thammapalo S, Morrison AC, Libraty DH, Green S, Scott TW, 2008. Spatial and temporal clustering of dengue virus transmission in Thai villages. *PLoS Med* 5: e205.
20. Endy TP, Nisalak A, Chunsuttiwat S, Libraty DH, Green S, Rothman AL, Vaughn DW, Ennis FA, 2002. Spatial and temporal circulation of dengue virus serotypes: a prospective study of primary school children in Kamphaeng Phet, Thailand. *Am J Epidemiol* 156: 52–59.
21. Klungthong C, Gibbons RV, Thaisomboonsuk B, Nisalak A, Kalayanaroj S, Thirawuth V, Nutkamhang N, Mammen MP, Jarman RG, 2007. Dengue virus detection using whole blood for Reverse Transcriptase PCR and virus isolation. *J Clin Microbiol* 45: 2480–2485.
22. Lanciotti RS, Calisher CH, Gubler DJ, Chang GJ, Vorndam AV, 1992. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. *J Clin Microbiol* 30: 545–551.
23. Innis BL, Nisalak A, Nimmannitya S, Kusalerdchariya S, Chongswasdi V, Suntayakorn S, Puttisri P, Hoke CH Jr, 1989. An enzyme-linked immunosorbent assay to characterize dengue infections where dengue and Japanese encephalitis co-circulate. *Am J Trop Med Hyg* 40: 418–427.
24. Sadon N, Delers A, Jarman RG, Klungthong C, Nisalak A, Gibbons RV, Vassilev V, 2008. A new quantitative RT-PCR method for sensitive detection of dengue virus in serum samples. *J Virol Methods* 153: 1–6.
25. Clarke DH, Casals J, 1958. Techniques for hemagglutination and hemagglutination inhibition with arthropod-borne viruses. *Am J Trop Med Hyg* 7: 561–573.
26. Russell PK, Nisalak A, Sukhavachana P, Vivona S, 1967. A plaque reduction test for dengue virus neutralization antibodies. *J Immunol* 99: 285–290.
27. Libraty DH, Endy TP, Houn H, Green S, Kalayanaroj S, Suntayakorn S, Chansiriwongs W, Vaughn DW, Nisalak A, Ennis FA, Rothman AL, 2002. Differing influences of virus burden and immune activation on disease severity in secondary dengue-3 virus infections. *J Infect Dis* 185: 1213–1221.
28. Vaughn DW, Green S, Kalayanaroj S, Innis BL, Nimmannitya S, Suntayakorn S, Endy TP, Raengsakulrach B, Rothman AL, Ennis FA, Nisalak A, 2000. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. *J Infect Dis* 181: 2–9.